The calcium-sensing receptor directly regulates proximal tubular functions

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Abstract: Capasso et al. show a role of the calcium-sensing receptor (CaSR) in enhancing proximal tubular fluid absorption and urinary acidification by stimulation of luminal Na(+)/H(+) exchanger (NHE) activity. NHE3 is required for sodium and fluid absorption, and its activity is coupled to passive reabsorption of a major fraction of calcium through the paracellular route. These data shed new light on the regulation of the kidney by the CaSR and whether it directly affects proximal tubular functions.

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The Calcium-sensing receptor directly regulates proximal tubular functions

(Commentary on: Calcium sensing receptor modulates fluid reabsorption and H^+-secretion in the proximal tubule by Capasso et al.)

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ABSTRACT

The Calcium sensing receptor (CaSR) plays a major role in determining circulating PTH levels and thereby systemic calcium levels. The CaSR is also highly expressed in kidney including the proximal tubule where it has been linked to regulating phosphate reabsorption. In an article in this issue of Kidney International, a direct role of the CaSR in enhancing proximal tubular fluid absorption and urinary acidification by stimulating apical Na^+/H^+ exchange activity is shown. Microperfusion experiments in vitro and micropuncture measurements in vivo using mice and rats demonstrate that stimulation of the CaSR increases fluid absorption. In CaSR KO mice this effect is abolished shedding also new light on the controversy whether the CaSR is expressed in the proximal tubule. The effect on fluid absorption is mediated by a stimulation of luminal Na^+/H^+ exchanger activity. The major NHE isoform in the proximal tubule, NHE3, is required for sodium and fluid absorption allowing for passive reabsorption of a amjor fraction of filtered calcium through the paracellular route. Thus, the CaSR may play an important role in regulating proximal tubular function affecting phosphate, fluid, salt. and calcium reabsorption.

The calcium sensing receptor (CaSR), a G protein–coupled receptor (GPCR), plays a critical role in the regulation of total body calcium homeostasis by modulating PTH secretion by the parathyroid glands. Stimulation of the CaSR by high extracellular ionized calcium concentrations suppresses PTH secretion whereas low calcium concentrations permit PTH synthesis and secretion [1]. The molecular identification of the CaSR in 1993 by E. Brown and S. Hebert paved the way for the development of a novel class of drugs, calcimimetics, that act on the CaSR and suppress parathyroid hormone [2]. These drugs are now widely used for the treatment of various forms of hyperparathyroidism including secondary hyperparathyroidism in CKD patients [3]. However, the CaSR is expressed in various other organs outside the parathyroid gland, including bone cells, cartilage, along the intestinal tract, vasculature, and in kidney. In these organs, the CaSR has been linked to many functions that are involved in maintaining body calcium homeostasis as well as local processes such as fluid movements in the colon or calcification of vessels.

The direct role of the CaSR in the kidney has only been partially elucidated. Two recent studies demonstrated a role of the CaSR in the regulation of paracellular calcium absorption in the thick ascending limb of the loop of Henle using kidney-specific CaSR KO mice or parathyroidectomized rat models [4-5]. In the TAL, the CaSR is highly expressed and regulates the NKCC2 cotransporter, target of loop diuretics, and claudin 14, a regulator of paracellular cation permeability. Earlier studies demonstrated a role of high luminal calcium concentrations for the regulation of water transport and proton secretion along the collecting duct consistent with an important role of the CaSR in the prevention of high luminal calcium concentrations and calcium precipitations [6-7].

The expression and function of the CaSR in the proximal tubule has been controversial. Immunolocalization and mRNA studies as well as functional evidence from isolated perfused proximal tubules indicated that the CaSR is expressed in the brush border membrane of the proximal tubule [8-9] whereas more recent data suggested that in mouse, rat and human kidney CaSR expression is mostly restricted to the thick ascending limb of the loop of Henle [4]. The role of the CaSR in the proximal tubule has been mostly linked to the regulation of phosphate reabsorption [8-9]. Stimulation of the CaSR in the presence of PTH prevents the phosphaturic effect of PTH [8]. High phosphate intake or elevated PTH reduce CaSR expression in the proximal tubule [9], thereby possibly limiting the antagonistic effect of the CaSR on the phosphaturic action of PTH.

In this issue of Kidney International Capasso and colleagues report a novel function of the CaSR in the kidney, the regulation of fluid reabsorption and proton secretion by the proximal tubule [10]. Using a combination of pharmacological tools and genetic models they report two key findings. First, stimulation of the CaSR with increasing luminal calcium concentrations enhanced fluid absorption by the proximal tubule both in *in vitro* microperfusion experiments as well as in *in vivo* micropuncture studies in mouse and rat, respectively. The same effect could be observed with a calcimimetic agent stimulating the CaSR whereas in proximal tubules from CaSR deficient mice no stimulation of fluid absorption was observed. The use of the genetic model supports the presence of functional CaSRs in the proximal tubule. Second, activation of the CaSR caused increased sodium-proton exchanger activity in isolated proximal tubules.

Sodium-proton exchange in the proximal tubule is mediated mostly by the Na^+/H^+-exchanger isoform 3 (NHE3) localized in the brush border membrane. There, NHE3 mediates the excretion of intracellular protons in exchange for luminal Na^+ ions, a major transport pathway for the reabsorption of sodium in the proximal tubule. Protons excreted by this pathway react with filtered bicarbonate and form H_2CO_3 eventually leading to CO_2 that crosses the brush border membrane into proximal tubular cells where it is hydrated to form bicarbonate. This bicarbonate leaves the proximal tubule cells together with three sodium ions through the NBCe1 Na^+/bicarbonate cotransporter into blood. This process causes an acidification of the luminal fluid from initially pH 7.4 to approximately pH 6.8 by the end of the proximal tubule. In addition, the accumulation of sodium ions (together with other reabsorbed solutes) increases interstitial osmolarity which together with a lumen-negative potential drives water, chloride and bicarbonate through the paracellular pathway (so-called 'solvent drag') helping in the reabsorption of approximately 70 % of all filtered salt and water along the proximal tubule. However, also calcium moves through the paracellular pathway accounting for approximately 60-70 % of all filtered calcium.

Stimulation of the CaSR in the proximal tubule increasing NHE3 activity may help reabsorbing calcium by at least two different mechanisms. In the proximal tubule, regulation of NHE3 is important for fluid absorption and solvent drag mediated calcium absorption (Figure 1). Enhanced NHE3 activity increases fluid absorption whereas loss of NHE3 causes renal salt and water loss. Accordingly, mice lacking NHE3 have a higher fractional excretion of calcium despite having elevated $1,25(OH)_2$ vitamin D_3 levels and near normal acid-base balance [11]. Clinically, the use of thiazide diuretics is well known to reduce renal calcium excretion by enhancing tubular calcium reabsorption. Even though the mechanism has not been fully clarified, this effect is preserved in mice lacking Trpv5, the channel responsible for distal calcium absorption and can be overcome by adding salt to the diet which reduces proximal tubular NHE3 activity [12]. Thus, CaSR mediated stimulation of proximal tubular fluid absorption through cellular and paracellular routes increases paracellular calcium absorption. Whether the CaSR in the proximal tubule plays a role in the thiazide induced hypocalcuria would be an interesting question.

The second mechanism how the CaSR in the proximal tubule may enhance renal calcium absorption may relate to urinary acidification. The fall in luminal pH

resulting from the removal of bicarbonate from the ultrafiltrate increases the fraction of ionized calcium along the proximal tubule. Enhanced NHE3 activity increases bicarbonate reabsorption and acidifies urine. However, the luminal pH at the end of the proximal tubule is further modified by the activity of bicarbonate absorbing and proton secreting processes in the thick ascending limb of the loop of Henle and the late distal convoluted tubule. Whether a lower urinary pH at the end of the proximal tubule is sufficient to allow for increased rates of calcium absorption by TRPV5 in the late distal convoluted tubule and connecting segment remains an open question.

How is the CaSR in the proximal tubule regulated and stimulated ? Regulation of expression has been shown for dietary phosphate intake and PTH [9]. Changes in ligand concentration or affinity may also play a more important role in the acute changes in CaSR activity. Luminal calcium concentrations depend mostly on the filtered calcium load and thereby reflect plasma calcium concentrations. Besides fluctuations in the filtered calcium, the affinity of the CaSR to calcium can be modulated by a variety of factors including pH, salinity, or aromatic amino acids. Thus, the CaSR may not only serve the regulation of calcium reabsorption but may also be involved in regulating the renal response to changes in acid-base status or salt intake. Both processes have been known for a long time to be linked also to calcium homeostasis. Whether the CaSR in the proximal tubule is a molecular link remains to be clarified.

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Disclosure

No financial conflicts.

FIGURE LEGEND

Figure 1

Schematic proximal tubular cell mediating reabsorption of bicarbonate and sodium. Carbonic anhydrases (CAII and CAIV) catalyze the formation of H2CO3 and HCO3-, respectively, whereas intracellular protons are exchanged for luminal sodium by NHE3. Both bicarbonate and sodium are extruded into the interstitium and blood by te basolateral Na+/HCO3 cotransporter NBCe1. Accumulation of solutes and electrolytes at the basolateral side of rpoximal tubular cells creates an osmotic driving force for the transcellular and paracellular movement of electrolytes and calcium from the lumen to the interstitium ('solvent drag'). CaSRs expressed on the luminal side of proximla tubular cells stimulate NHE3 activity and may thereby enhance calcium absorption via the paracellular route.

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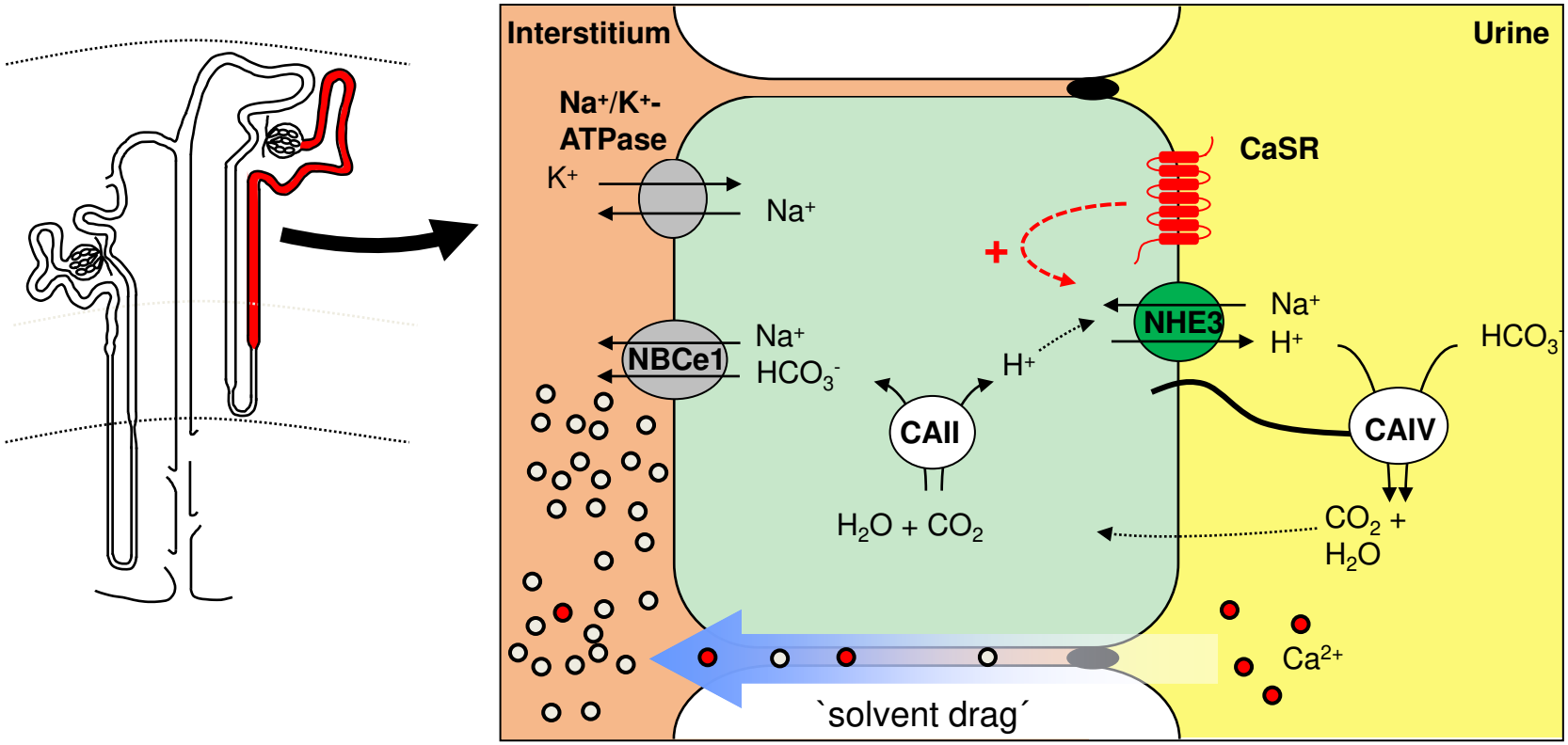
Interstitium
Urine
Na+/K+-
ATPase
K+
Na+
CaSR
+
NHE3
Na+
H+
HCO3-
NBCe1
Na+
HCO3-
H+
CAII
CAIV
H2O + CO2
CO2 +
H2O
Ca2+
`solvent drag´